Fisheries and Oceans Canada | Pêches et Océans Canada
Science | Sciences
Maritimes Region
Canadian Science Advisory Secretariat
Science Response 2014/043

DISTINCTIVENESS AND STATUS OF THE SAINT JOHN RIVER POPULATION OF SHORTNOSE STURGEON (*ACIPENSER BREVIROSTRUM*)

Context

Science advice on the genetic distinctiveness and population status of the Saint John River (SJR) population of Shortnose Sturgeon (*Acipenser brevirostrum*) was requested by Fisheries Resource Management in the National Capital Region. This information may be used to inform a possible decision by the United States (US) government to delist the Saint John River population of Shortnose Sturgeon from the US *Endangered Species Act* (ESA), i.e., through recognition of the SJR population of Shortnose Sturgeon as a Distinct Population Segment and concurrence with the Canadian *Species at Risk Act* (SARA) listing of Shortnose Sturgeon as a species of Special Concern. Specifically, the following questions were asked:

- Is the Saint John River population of Shortnose Sturgeon sufficiently genetically distinct from all other populations of Shortnose Sturgeon as to meet the criteria for recognition as a Designatable Unit (Canada) and a Distinct Population Segment (USA)?
- What is known about the present status of the Saint John River Shortnose Sturgeon population?

DFO Science was also asked to provide recommendations on what work would need to be done to answer these questions if they cannot be fully addressed with the information available.

Given the short timeframe to provide a response, DFO's Science Response Process was used.

This Science Response Report results from the Science Response Process of 18 August, 2014, on the Status of Saint John River Population of Shortnose Sturgeon (*Acipenser brevirostrum*).

Background

Shortnose Sturgeon is a long-lived (oldest female caught = 67 years, oldest male caught = 32 years) anadromous fish species found on the east coast of North America from New Brunswick, Canada, to Florida, US. The only known Shortnose Sturgeon population in Canada is found in the Saint John River, in southwest New Brunswick (Figure 1). Shortnose Sturgeon was evaluated as Special Concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 1980, and it was re-assessed and evaluated as Special Concern in 2005. The reason for this determination was as follows:

> *This is an anadromous species restricted to a single river system in Canada where spawning fish require unhindered access to freshwater spawning sites; but the population may have been divided since 1967 by the Mactaquac Dam. These large, slow growing, late maturing fish are conservation dependent. There is some risk to the species through mortality from hydroelectric facilities, by-catch in alewife and shad fisheries, and poaching. However, there is no immediate threat that would lead to elimination of the population in a very short period of time. (COSEWIC 2005)*

Shortnose Sturgeon was listed as Special Concern on Schedule 1 of the Canadian *Species at Risk Act* in 2009 and a Management Plan is under development as required by the Act. Shortnose Sturgeon is listed at the species level (Canada-US) as Endangered under the US ESA. However, the National Marine Fisheries Service recognizes 19 distinct population segments occurring in New Brunswick,

Canada (1), Maine (2), Massachusetts (1), Connecticut (1), New York (1), New Jersey/Delaware (1), Maryland/Virginia (1), North Carolina (1), South Carolina (4), Georgia (4) and Florida (2) (NMFS 1998).

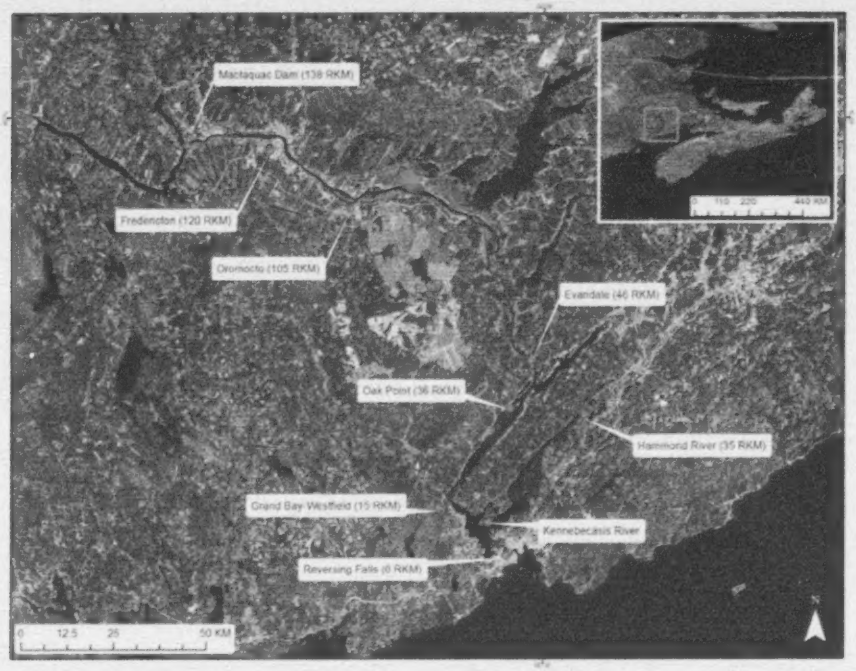

Figure 1. Saint John River from the Reversing Falls at its mouth to Mactaquac Dam. RKM refers to river kilometers, i.e. distance in km from the mouth of the river. This map was created by the Oceans and Coastal Management Division of Fisheries and Oceans Canada based on a map in COSEWIC (2005).

Analysis and Response

Population Distinctiveness

Criteria for Determining Population Distinctiveness

Under the US ESA, the definition of species extends not only to formally recognized species, but also to any *distinct population segment* (DPS) within a vertebrate species. A joint policy issued by the National Marine Fisheries Service (NMFS) and the United States Fish and Wildlife Service (USFWS) (USFWS and NMFS 1996) utilizes two criteria, *discreteness* and *significance*, in order to determine whether a population or group of populations merits recognition as a DPS. The criteria, and the kinds of evidence used to assess them, are listed in Table 1. The NMFS initiated a status review of the Shortnose Sturgeon in 2007 with the intent to incorporate latest technologies and availability of molecular data to identify DPSs within the species (SSSRT 2010).

Under the Canadian SARA, wildlife species afforded protection can also include geographically or genetically distinct populations. COSEWIC uses the term designatable unit (DU) to define units within species that merit recognition as wildlife species. DUs are defined using principles of *discreteness* and *significance* closely similar to those used to recognize DPSs, along with similar types of supporting evidence (Table 1).

Table 1. Criteria and supporting evidence used for defining Distinct Population Segment (US)[1, 2] and Designatable Units (Canada)[3].

Criteria	United States (DPS)	Canada (DU)
Discreteness	Evidence of genetic distinctiveness (e.g. inherited traits, neutral genetic markers).	1. Evidence of genetic distinctiveness including, but not limited to, inherited traits (e.g. morphology, life history, behaviour) and/or neutral genetic markers (e.g. allozymes, DNA microsatellites, DNA restriction fragment length polymorphisms (RFLPs), DNA sequences).
	Markedly separated from other populations of the same taxon.	2. Natural disjunction between substantial portions of the species' geographic range, such that movement of individuals between separated regions has been severely limited for an extended period of time and is not likely in the foreseeable future and where the disjunction is likely to favour the evolution of local adaptations.
	Delimited by international governmental boundaries.	3. Occupation of differing eco-geographic regions that are relevant to the species and reflect historical or genetic distinction, as may be depicted on an appropriate ecozone or biogeographic zone map (Figs. 1 - 3). Some dispersal may occur between regions, but it is insufficient to prevent local adaptation.
Significance	Evidence that the discrete population segment differs markedly from other populations of the species in its genetic characteristics.	1. Evidence that the discrete population or group of populations differs markedly from others in genetic characteristics thought to reflect relatively deep intraspecific phylogenetic divergence. Such differences would typically be manifested as qualitative genetic differences at relatively slow-evolving markers (e.g. fixed differences in mitochondrial or nuclear DNA sequences or fixed differences in alleles at multiple nuclear loci). Quantitative (frequency) differences of shared alleles, especially for rapidly-evolving markers such as microsatellites, generally would not be sufficient to meet this criterion.
	Persistence in an ecological setting unusual or unique for the taxon.	2. Persistence of the discrete population or group of populations in an ecological setting unusual or unique to the species, such that it is likely or known to have given rise to local adaptations.
	Evidence that the discrete population segment represents the only surviving natural occurrence of a taxon that may be	3. Evidence that the discrete population or group of populations represents the only surviving natural occurrence of a

Criteria	United States (DPS)	Canada (DU)
	more abundant elsewhere as an introduced population outside its historic range.	species that is more abundant elsewhere as an introduced population outside of its historical range.
	Evidence that loss of the discrete populations segment would result in a significant gap in the range of a taxon.	4. Evidence that the loss of the discrete population or group of populations would result in an extensive gap in the range of the species in Canada.
Status	If a population segment is considered a distinct population its evaluation for endangered or threatened status will be based on the Act's definitions of those terms.	

[1] U.S. Fish and Wildlife, National Marine Fisheries Service. 1996. Distinct Population Segment (61 Fed. Reg. 4722, Feb. 7, 1996.

[2] Fay JJ, Nammack M. 1996. Fish and Wildlife Service's Division of Endangered Species. USA Federal Register, Department of the Interior, Fish and Wildlife Service, National Marine Fisheries Service, National Oceanic and Atmospheric Association, Commerce. Vol 61, No. 26.

[3] COSEWIC (Committee on the Status of Endangered Wildlife in Canada) 2011. Note: evidence guidelines are reproduced verbatim from COSEWIC (Accessed September 2014).

Genetic Studies on Shortnose Sturgeon that Bear on the DPS/DU Status of the Saint John River Population

Wirgin et al. 2010

The intent of this study was to use mitochondrial DNA (mtDNA) data to assess the genetic discreteness of Shortnose Sturgeon populations and "inform the delineation of DPSs". This study combined mtDNA data from new samples with previously published data (Grunwald et al. 2002; Waldman et al. 2002; Wirgin et al. 2005), and, therefore, represents the most comprehensive study conducted on mtDNA variation in Shortnose Sturgeon to date.

Shortnose Sturgeon samples were analyzed from 14 of the 19 distinct population segments recommended by the Shortnose Sturgeon Recovery Team (SSRT) (NMFS 1998) (Table 2). A 1.1 kilobase pair DNA sequence encompassing the mtDNA control region was amplified using Shortnose Sturgeon specific primers and sequenced. The mtDNA control region was targeted because this portion of the mitochondrial genome evolves relatively rapidly, and hence is more likely to reveal sequence variations that can be used to assess genetic differentiation of populations.

Table 2. Sample locations, number of specimens, mtDNA haplotypes detected, haplotype diversity index and mean number of pairwise differences with Shortnose Sturgeon collections (reproduced from Wirgin et al. 2010).

River	Number of Specimens	Number of Haplotypes	Haplotype Diversity	Mean Number of Pairwise Differences
Saint John	42	8	0.696	1.830
Penobscot	44	8	0.853	4.846
Kennebec	54	8	0.781	4.870
Androscoggin	48	8	0.812	4.836
Connecticut	46	4	0.660	3.109
Hudson	56	9	0.777	4.523
Delaware	57	8	0.672	2.783
Chesapeake	39	6	0.719	3.101
Cape Fear	5	5	1.000	3.800
Winyah	46	13	0.853	3.033
Santee	4	2	0.500	3.000
Marion	41	5	0.672	2.532
Cooper	62	6	0.783	3.099
Savannah	25	7	0.800	2.110
Ogeechee	53	11	0.857	3.186
Altamaha	69	10	0.862	3.280

Among 691 Shortnose Sturgeon analyzed, a total of 35 polymorphic nucleotide sites were observed, resulting in 38 haplotypes, or sequence variants (Table 3). Haplotypes typically occurred in multiple rivers, but often showed marked frequency differences among rivers. Northern populations, defined as those from the Kennebec system northward to the Saint John River (SJR), had 12 haplotypes, of which eight were restricted to this portion of the species range. With the exception of haplotypes C and M all haplotypes in Southern regions were specific to that area (Table 3); thus, the broad pattern was one of markedly different haplotype distributions on regional scales.

Table 3. Frequencies of mtDNA control region haplotypes in Shortnose Sturgeon collections analysed in this study (reproduced from Wirgin et al. 2010).

Haplotype	Saint John	Penobscot	Kennebec	Androscoggin	Connecticut	Hudson	Delaware	Chesapeake	Cape Fear	Winyah	Santee	Marion	Cooper	Savannah	Ogeechee	Altamaha	Total
A						1	30	19									50
B					19	1	11	7									38
C							8	4	1	15	1	3	13	3	14	8	70
D							1										1
E							1	4									5
F						22	1	2									25
G									1					6	2	2	11
H										1		5	4		1	15	26
I	1	3	2	3													9
J						1											1
K	1																1
L	1	7	5	6		12	4	3									38
M						8	1			4				1	5	14	34
N										1				1	5	2	9
O										4		12	10	4	9	6	45
P		12	21	15	18												66
Q									1	3	3	20	20		3	11	61
R										7			14	9	10	8	48
S	16	3	1	2													22
T	1	6	8														15
U	1				8	6											15
V						2											2
W															1	2	3
X	4	5	11	7													27
Y						3											3
Z	17	7	5	12	1												42
AA												1	1				2
BB									1							1	2
CC		1		1													2
DD				2													2
EE										5					1		6
FF										1							1
GG										1							1
HH										1							1
II										2							2
JJ			1														1
M/R														1			1
KK										1					2		3
N	42	44	54	48	46	56	57	39	5	46	4	41	62	25	53	69	691

Within the northern group of rivers, the SJR population showed substantial differences in haplotype frequencies relative to those in the other three rivers, the Penobscot, Kennebec and Androscoggin, which were genetically similar to each other (Table 3). The dominant haplotypes in the SJR, 'S' and 'Z', were present at frequencies of 38% and 40%, respectively. The average frequencies of these two

haplotypes in the three other northern rivers were 4% and 16%, respectively. Conversely the modal haplotype in the three Maine rivers was 'P', present at an average frequency of 33%, was not observed in the SJR. Not surprisingly, the differences in haplotype frequencies between the SJR and the Maine populations were found to be highly significant (Monte Carlo based Chi-square test; P = 0.0000; Table 4). By contrast, haplotype frequencies in the Penobscot, Kennebec and Androscoggin Rivers did not differ significantly from each other. Across the remainder of the species range, haplotype frequencies were usually, but not invariably, different between geographically proximate rivers (Table 4).

Table 4. Chi-square comparisons of significance of differences in frequencies of mtDNA control region haplotypes among Shortnose Sturgeon collections (uncorrected p values in parentheses; sig after Bonferroni correction = 0.0005 (reproduced from Wirgin et al. 2010).

	Penobscot	Kennebec	Androscoggin	Connecticut	Hudson	Delaware	Chesapeake	Winyah	Marion	Cooper	Savannah	Ogeechee	Altamaha
Saint John	37.22 (0.0000)	54.85 (0.0000)	37.91 (0.0000)	80.65 (0.0000)	90.73 (0.0000)	95.72 (0.0000)	78.00 (0.0000)	88.00 (0.0000)	83.00 (0.0000)	104.00 (0.0000)	67.00 (0.0000)	95.00 (0.0000)	111.00 (0.0000)
Penobscot		7.92 (0.4524)	10.10 (0.2571)	90.00 (0.0000)	82.06 (0.0000)	90.65 (0.0000)	35.60 (0.0000)	90.00 (0.0000)	85.00 (0.0000)	106.00 (0.0000)	32.96 (0.0000)	97.00 (0.0000)	113.00 (0.0000)
Kennebec			17.10 (0.0240)	57.63 (0.0000)	95.88 (0.0000)	102.10 (0.0000)	85.30 (0.0000)	100.00 (0.0000)	95.00 (0.0000)	116.00 (0.0000)	79.00 (0.0000)	107.00 (0.0000)	123.00 (0.0000)
Androscoggin				57.56 (0.0000)	87.90 (0.0000)	95.33 (0.0000)	78.91 (0.0000)	94.00 (0.0000)	85.28 (0.0000)	110.00 (0.0000)	73.00 (0.0000)	101.00 (0.0000)	117.00 (0.0000)
Connecticut					84.32 (0.0000)	74.81 (0.0000)	64.40 (0.0000)	92.00 (0.0000)	87.00 (0.0000)	108.00 (0.0000)	71.00 (0.0000)	99.00 (0.0000)	115.00 (0.0000)
Hudson						86.08 (0.0000)	69.96 (0.0000)	91.64 (0.0000)	97.00 (0.0000)	118.00 (0.0000)	76.83 (0.0000)	95.69 (0.0000)	104.41 (0.0000)
Delaware							5.80 (0.6039)	78.65 (0.0000)	89.03 (0.0000)	99.16 (0.0000)	69.35 (0.0000)	86.27 (0.0000)	106.09 (0.0000)
Chesapeake								72.28 (0.0000)	73.14 (0.0000)	88.10 (0.0000)	56.80 (0.0000)	79.26 (0.0000)	96.44 (0.0000)
Winyah									51.11 (0.0000)	34.81 (0.0001)	28.32 (0.0024)	15.85 (0.2992)	38.24 (0.0000)
Marion										16.97 (0.0023)	46.88 (0.0000)	49.05 (0.0000)	37.17 (0.0000)
Cooper											34.39 (0.0001)	31.61 (0.0000)	34.53 (0.0000)
Savannah												17.65 (0.0575)	32.96 (0.0000)
Ogeechee													27.54 (0.0008)

The Saint John River population exhibited the lowest mean number of pairwise nucleotide differences between individuals, (1.83) compared to values in the three Maine rivers of 4.85-4.87 (Table 2). The greater level of genetic diversity in the Maine rivers suggests larger long-term effective population sizes in that region.

Pairwise Φ_{ST} values, a measure of genetic differentiation incorporating both haplotype frequency differences and the extent of sequence divergence between haplotypes, approached zero in comparisons between the Penobscot, Kennebec and Androscoggin Rivers (Table 5). By contrast, Φ_{ST} between the Penobscot and SJR was 0.213, a relatively high value, considering that the 'global' Φ_{ST} across all rivers was 0.331. Using the haplotype data and two different methods, one based directly on

Φ_{ST} and the other on a coalescent approach, Wirgin et al. (2010) derived estimates of female mediated gene flow between the Penobscot and Saint John Rivers of 1.9 and 2.1 effective migrants per generation, respectively. The Φ_{ST} based estimate is subject to the assumption that the populations in question are at long-term genetic equilibrium, and that the pattern of migration corresponds to Wright's 'Island' model, neither of which is likely to hold. Nonetheless, the similarity of the two estimates suggests that they are reasonably robust. However, the estimates pertain to 'long-term' migration, and not to contemporary, or even recent migration rates; moreover, the inferred migration could at least partly reflect historical colonization patterns from a shared glacial refugium (Waldman et al. 2002).

Table 5. Pairwise Φ_{ST} values (above the diagonal) and estimates of female mediated gene flow (below diagonal) (reproduced from Wirgin et al. 2010).

Locale	Saint John	Penebscot	Kennebec	Androscoggin	Connecticut	Hudson	Delaware	Chesapeake	Winyah	Marion	Cooper	Savannas	Ogeechee	Altamaha
Saint John		0.213	0.291	0.243	0.377	0.235	0.446	0.418	0.278	0.551	0.412	0.505	0.345	0.326
Penobscot	2.85		-0.0001	-0.0008	0.063	0.189	0.224	0.194	0.316	0.492	0.421	0.426	0.361	0.365
Kennebec	1.22	####		0.003	0.068	0.241	0.236	0.212	0.365	0.521	0.461	0.463	0.407	0.412
Androscoggin	1.56	####	####		0.064	0.210	0.227	0.199	0.345	0.507	0.442	0.447	0.387	0.391
Connecticut	0.827	7.42	6.90	7.31		0.254	0.271	0.236	0.416	0.587	0.502	0.535	0.450	0.449
Hudson	1.63	2.14	1.58	1.89	1.47		0.322	0.266	0.221	0.328	0.262	0.314	0.238	0.236
Delaware	0.622	1.73	1.323	1.70	1.35	1.05		-0.011	0.437	0.614	0.535	0.585	0.489	0.480
Chesapeake	0.697	2.07	1.529	2.02	1.62	1.38	####		0.412	0.586	0.506	0.564	0.463	0.453
Winyah	1.30	1.08	0.798	0.950	0.701	1.77	0.644	0.715		0.284	0.120	0.172	0.038	0.044
Marion	0.407	0.515	0.438	0.487	0.351	1.03	0.314	0.353	1.26		0.054	0.292	0.197	0.148
Cooper	0.787	0.687	0.561	0.631	0.497	1.41	0.435	0.489	3.67	8.70		0.116	0.052	0.029
Savannah	0.490	0.673	0.551	0.618	0.435	1.09	0.355	0.387	2.41	1.21	3.83		0.038	0.086
Ogeechee	0.948	0.855	0.696	0.793	0.610	1.60	0.523	0.580	12.5	2.03	9.10	12.8		0.014
Altamaha	1.04	0.869	0.683	0.780	0.614	1.62	0.542	0.604	10.9	2.88	16.8	5.33	35.8	

Female mediated gene flow estiamtes, $N_eM_f = ((1/\Phi_{ST}-1)2)$, are illustrated below the diagonal. Number signs indicate values of infinity.

A UPGMA (Unweighted Pair Group Method with Arithmetic Mean) tree based on Nei's genetic distances calculated from the haplotype data showed regional clustering of shortnose populations largely concordant with the Acadian, Virginian and Carolinian provinces (Figure 2). An exception was grouping of the Saint John and Hudson rivers, which was regarded by the authors as an anomaly, as suggested by the low overlap of haplotype identities and highly significant differences in haplotype frequencies between the two collections. The Hudson-SJR grouping was thought to be caused by sequence similarity between predominant haplotypes F and L in the Hudson and haplotypes S and Z in the SJR. The authors further speculated that inability of the dendrogram to distinguish these two rivers may be hampered by insufficient sequence data producing low resolution, and high levels of homoplasy (parallel or convergent mutations in the mutationally active control region). Notably, bootstrap support for the Hudson-SJR group was low (<50%).

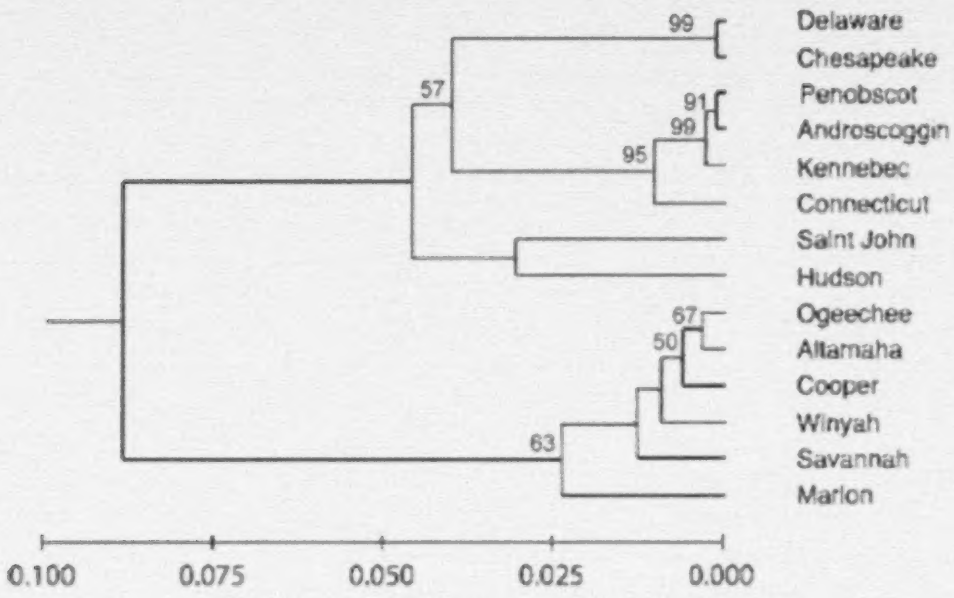

Figure 2. UPGMA tree of the population genetic distances for mtDNA control region sequence data from Shortnose Sturgeon from 13 Atlantic Coast Rivers and estuaries. Nodes without bootstrap values indicate values of <50 (reproduced from Wirgin et al. 2010).

Wirgin et al. (2010) conducted analyses of molecular variance (AMOVA) to investigate the proportions of genetic variation explained by grouping Shortnose Sturgeon populations in different ways (Table 6). When populations were grouped into three units corresponding to biogeographic provinces (Acadian, Virginian, Carolinian), the percentage of genetic variation explained by differences among groups and among populations within groups was 27.8% and 10.8%, respectively. The authors also considered various scenarios in which the populations were aggregated in 6, 9 or 10 groups. Notably, all of these latter grouping models specified the SJR as a separate group. The percentage of genetic variation explained by among group differences was similar for all grouping scenarios, 33.6-34.4%. The genetic variation among populations within groups tended to decline as the number of groups increased, and was at a minimum (0.25%) for one scenario involving 10 population groups. This is to be expected, since the Wirgin et al. study also showed that mtDNA variation in Shortnose Sturgeon follows a pattern of isolation by distance (IBD), in which genetic distance between populations is correlated with the geographic distance between them. Nonetheless, the fact that the most conservative (fewest groups) scenario that included SJR as a separate group (for a total of six groups) resulted in the maximum value for among group variation (34.4%) suggests a deeper and more fundamental division in the northern/Acadian group than can be explained solely by IBD. Unfortunately, the authors did not evaluate a four group scenario in which the Acadian Province was split into Canadian and US portions (SJR, Maine rivers, Virginian, Carolinian).

Table 6. Hierarchical structuring of mtDNA control region sequence variation among Shortnose Sturgeon collections using AMOVA (reproduced from Wirgin et al. 2010).

Model	Source of Variation of Variation	Percentage	*p*
3 groups (Acadian, Virginian, Carolinian Provinces)	Among groups	27.8	0.001
	Among pops. within group	10.8	0.000
	Within pops.	61.4	0.000
6 groups (Saint John, Penobscot-Kennebec, Connecticut, Hudson, Delaware-Chesapeake, Winyah-Marion-Cooper-Savannah-Ogeechee-Altamaha)	Among groups	34.4	0.000
	Among pops. within group	3.53	0.000
	Within pops.	62.1	0.000
9 groups (Saint John, Penobscot-Kennebec, Connecticut, Hudson, Delaware-Chesapeake, Winyah, Marion-Cooper, Savannah, Ogeechee-Altamaha)	Among groups	34.0	0.000
	Among pops. within group	0.77	0.125
	Within pops.	65.3	0.000
9 groups (Saint John, Penobscot-Kennebec, Connecticut, Hudson, Delaware-Chesapeake, Winyah, Marion-Cooper, Savannah-Ogeechee, Altamaha)	Among groups	33.6	0.000
	Among pops. within group	0.91	0.079
	Within pops.	65.5	0.000
10 groups (Saint John, Penobscot-Kennebec, Connecticut, Hudson, Delaware-Chesapeake, Winyah, Marion-Cooper, Savannah, Ogeechee, Altamaha)	Among groups	33.6	0.000
	Among pops. within group	0.76	0.171
	Within pops.	65.7	0.000
10 groups (Saint John, Penobscot-Kennebec, Connecticut, Hudson, Delaware-Chesapeake, Winyah, Marion, Cooper, Savannah, Ogeechee-Altamaha)	Among groups	34.2	0.000
	Among pops. within group	0.25	0.419
	Within pops.	65.5	0.000

Significance levels are base on 1000 permutations.

King et al. 2014 (and King et al. 2013)

This study is the first to examine the population genetics of Shortnose Sturgeon using nuclear DNA markers. The reason that previous studies had exclusively targeted the maternally inherited, effectively haploid mitochondrial genome, is likely due to the fact that Shortnose Sturgeon are hexaploid. This complicates the interpretation of nuclear genetic data and makes it difficult or impossible to apply most commonly applied statistical methods for population genetic analysis. King et al. (2014) overcame these difficulties by applying a method previously validated on another polyploid sturgeon species (Rodzen and May 2002) in which microsatellite alleles were treated as pseudo-dominant markers. In practice this meant that they treated the occurrence of microsatellite alleles within individuals as binary presence/absence data; no attempt was made to try to infer levels of heterozygosity involving particular alleles or individual microsatellite loci, although they did estimate overall levels of heterozygosity across all loci. Using this approach they analyzed data on 11 microsatellite loci for 561 Shortnose Sturgeon from 17 populations, including 25 fish from the SJR (Table 7).

King et al. (2014) used two approaches to identify genetic clusters of individuals and populations. In one approach, principle coordinate analysis (PCoA) was carried out using distance measures calculated for all pairs of individuals based on the number of differences in their 'allelotype' profiles. The second approach consisted of Bayesian clustering using the program Structure. This method groups individuals into *k* genetic clusters irrespective of where the fish were sampled. Different values of *k* are evaluated, and the most probable value of *k* is chosen based on various criteria.

The two clustering methods yielded concordant views of broad-scale patterns of Shortnose Sturgeon genetic differentiation, in which three highly distinct population groups were identified: Southeast, mid-Atlantic and Northeast (Figure 3). The Northeast cluster comprised (from south to north) the

Merrimack, Androscoggin, Kennebec, Penobscot and Saint John River. The PCoA also highlighted the relatively greater divergence of the SJR and Merrimack from the three other rivers, which were all very genetically similar. The close relationship between the SJR and Merrimack implied by the range-wide PCoA is somewhat misleading, since the two populations appear distinct in a subsequent PCoA conducted only on Northeast populations (Figure 4).

The authors next ran the Structure analysis again, but separately on each of the three genetic clusters identified in the first Structure analysis. This approach is often used when the genetic structure of populations is thought to be hierarchical in nature, as is the case with Shortnose Sturgeon. The first round of Structure analysis identifies the 'deepest' genetic structure, and subsequent rounds test for shallower, but still significant, genetic divisions. In this case the second round Structure analysis clearly resolved three (sub) clusters within the large Mid-Atlantic cluster: Connecticut, Hudson, and Delaware-Chesapeake. These clusters were also clearly evident in a PCoA conducted on the Mid-Atlantic populations (Figure 4). In contrast, neither the second round of Structure nor a PCoA conducted on the Southeast populations further resolved any clear population groupings. However, both the second round Structure analysis and a PCoA identified three groups within the Northeast: Merrimack, Androscoggin-Kennebec-Penobscot, SJR, although the divisions between these 'second level' genetic clusters were not as deep as between those found in the Mid-Atlantic region (Figure 4).

King et al. (2014) computed two measures of pairwise difference between each population, Jaccard's distance metric, and Φ_{PT}, an analogue of F_{ST}, on the polyploid microsatellite data (Table 8). Statistical tests conducted on the Jaccard's distance values showed that differences were significant for the majority of pairwise comparisons, including all comparisons involving the SJR ($P = 0.000$ in all cases). Jaccard's distances and Φ_{PT} were highly correlated (Mantel test; $r = 0.98$, $P < 0.0001$). The authors also estimated the effective number of migrants for all pairs of populations, based on the pairwise Φ_{PT} values. The results supported all previous analyses, inasmuch as lower inferred migration rates were evident among the three major population groups, relative to within group rates of migration (Table 8).

A dendrogram based on the Φ_{PT} values using the neighbour-joining (NJ) method supported results obtained from the nuclear DNA data using other methods; it revealed the same three most strongly defined population clusters, with Southeast the most divergent of the three clusters, and showed the relative distinctiveness of the SJR and Merrimack within the Northeast cluster (Figure 5). Echoing the results of the range wide PCoA, the NJ tree grouped the SJR and Merrimack together. Most groupings in the dendrogram, including the SJR-Merrimack group, were supported by high bootstrap values. With regard to the anomalous SJR-Merrimack grouping, it should be noted that for data on highly polymorphic microsatellites, an N of 21 is sub-optimally small. The possibility that the Merrimack sample was not an adequate representation of genetic diversity in that population is heightened by the fact that it consisted of 21 male sturgeon collected at the same time and place.

The authors used AMOVAs to evaluate 11 different models of Shortnose Sturgeon population structure, in which populations were grouped in different ways. A variety of grouping models that either excluded SJR altogether, or included it as a separate group, resulted in slightly more genetic variance among groups (17%) than models that either used fewer than five groups, or included SJR within a larger group; this latter set of models all found 16% of the genetic variance among groups (Table 9).

Finally, King et al. (2014) conducted a test of particular practical significance: they tested the ability of genotype data for the 11 microsatellite markers to correctly assign Shortnose Sturgeon back to their source populations. The results revealed varying rates of correct assignment (Table 10). Most notably for this report, the correct assignment rate for SJR sturgeon was 80%. The remaining 20% (5 fish) assigned to populations in Maine. Of 86 sturgeon sampled in Maine rivers, one (approximately 1%) was assigned to the SJR population.

Table 7. Microsatellite allele (a.k.a. pseudodominant locus) counts, percentage of loci (alleles) polymorphic, number of private alleles, and number of common alleles across all loci for Shortnose Sturgeon (Acipenser brevirostrum) populations across the North American range. The analyses were conducted on the binary character matrix. [1]Number of different fragments. [2]Number of bands unique to a single population. [3]Number of common alleles with frequency ≤ 25%. (reproduced from King et al. 2014).

Population	Saint John	Penobscot	Androscoggin	Kennebec	Merrimack	Connecticut	Hudson	Delaware	Chesapeake Bay	Cape Fear	Winyah Bay	Santee-Cooper	Lake Marion	Edisto	Savannah	Ogeechee	Altamaha
Sample size (n)	25	39	23	24	22	47	45	39	34	3	47	42	33	33	34	35	36
[1]No. Alleles (loci)	118	131	126	130	105	121	152	134	127	55	119	111	95	112	113	112	107
Polymorphism (%)	65.2	72.4	69.1	70.7	58.0	66.9	84.0	74.0	70.2	22.7	65.2	60.8	51.9	61.9	61.9	60.8	59.1
[2]No. Private Alleles	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0
[3]No. Common Alleles (<=25%)	11	16	13	16	6	12	24	12	11	0	7	3	4	4	5	6	2

Table 8. Pair-wise Φ_{PT} among putative Shortnose Sturgeon populations (above diagonal) and estimates of the effective number of migrants per generation, N_em (below diagonal), for 17 collections of Shortnose Sturgeon surveyed at 11 polysomic microsatellite loci. Non-significant pair-wise Φ_{PT} probability values (H_0 = No genetic difference among populations; Φ_{PT} = 0) based on 10,000 permutations values are in bold italics. Cape Fear River sample is not included due to inadequate sample size. (reproduced from King et al. 2014).

Collection	Saint John	Penobscot	Androscoggin	Kennebec	Merrimack	Connecticut	Hudson	Delaware	Chesapeake Bay	Winyah Bay	Santee-Cooper	Lake Marion	Edisto	Savannah	Ogeechee	Altamaha
Saint John		0.068	0.077	0.068	0.100	0.191	0.162	0.175	0.155	0.253	0.289	0.269	0.269	0.278	0.280	0.277
Penobscot	3.43		*0.015*	*0.003*	0.065	0.116	0.094	0.107	0.095	0.189	0.219	0.207	0.189	0.201	0.205	0.203
Androscoggin	3.00	16.42		*0.013*	0.087	0.113	0.091	0.099	0.093	0.200	0.248	0.226	0.209	0.210	0.227	0.226
Kennebec	3.43	83.08	18.98		0.058	0.114	0.073	0.096	0.088	0.186	0.222	0.205	0.188	0.201	0.207	0.204
Merrimack	2.25	3.60	2.62	4.06		0.201	0.153	0.184	0.167	0.268	0.307	0.297	0.279	0.295	0.293	0.296
Connecticut	1.06	1.91	1.96	1.94	0.99		0.086	0.100	0.118	0.239	0.272	0.263	0.256	0.261	0.273	0.273
Hudson	1.29	2.41	2.50	3.17	1.38	2.66		0.067	0.075	0.179	0.217	0.208	0.188	0.196	0.210	0.201
Delaware	1.18	2.09	2.28	2.35	1.11	2.25	3.48		0.018	0.188	0.228	0.217	0.200	0.206	0.216	0.212
Chesapeake Bay	1.36	2.38	2.44	2.59	1.25	1.87	3.08	13.64		0.183	0.234	0.210	0.200	0.213	0.216	0.206
Winyah Bay	0.74	1.07	1.00	1.09	0.68	0.80	1.15	1.08	1.12		0.049	0.034	0.037	0.046	0.031	0.032
Santee-Cooper	0.62	0.89	0.76	0.88	0.56	0.67	0.90	0.85	0.82	4.85		0.044	0.043	0.043	0.046	0.069

Table 9. Hierarchical structuring of genetic variation was measured for numerous combinations of Shortnose Sturgeon collections using analysis of molecular variance (AMOVA). Significance levels of the variance components were based on 1000 permutations. (reproduced from King et al. 2014).

Model	Source of Variance	Percentage of Variance	Test Statistic	Value	Probability
17 individual populations	Among pops within groupings	16%	Φ_{PT}	0.164	0.001
	Within pops	84%			
1) 17 populations as 3 groupings	Among groupings	16%	Φ_{RT}	0.158	0.001
(NE; Mid-Atlantic; SE)	Among pops within groupings	5%	Φ_{PR}	0.057	0.001
	Within pops	79%	Φ_{PT}	0.206	0.001
2) 16 populations as 3 groupings	Among groupings	16%	Φ_{RT}	0.158	0.001
(#2 with SJ omitted)	Among pops within groupings	4%	Φ_{PR}	0.054	0.001
	Within pops	80%	Φ_{PT}	0.203	0.001
3) 17 populations as 5 groupings	Among groupings	16%	Φ_{RT}	0.164	0.001
(NE, CT, Hudson, DE/CB, SE)	Among pops within groupings	4%	Φ_{PR}	0.042	0.001
	Within pops	80%	Φ_{PT}	0.199	0.001
4) 16 populations as 5 groupings	Among groupings	17%	Φ_{RT}	0.166	0.001
(#3 with SJ omitted)	Among pops within groupings	3%	Φ_{PR}	0.037	0.001
	Within pops	80%	Φ_{PT}	0.196	0.001
5) 17 populations as 6 groupings	Among groupings	17%	Φ_{RT}	0.167	0.001
(#4 with SJ as a grouping)	Among pops within groupings	3%	Φ_{PR}	0.036	0.001
	Within pops	80%	Φ_{PT}	0.197	0.001

Model	Source of Variance	Percentage of Variance	Test Statistic	Value	Probability
6) 16 populations as 6 groupings (#4 omits SJ and has Merrimack as a grouping)	Among groupings	17%	Φ_{RT}	0.169	0.001
	Among pops within groupings	3%	Φ_{PR}	0.031	0.001
	Within pops	80%	Φ_{PT}	0.195	0.001
7) 17 populations as 7 groupings (#3 with SJ and Merrimack as groupings)	Among groupings	17%	Φ_{RT}	0.170	0.001
	Among pops within groupings	3%	Φ_{PR}	0.031	0.001
	Within pops	80%	Φ_{PT}	0.196	0.001
8) 17 populations as 6 groupings (#3 with Altamaha as grouping)	Among groupings	15%	Φ_{RT}	0.154	0.001
	Among pops within groupings	4%	Φ_{PR}	0.042	0.001
	Within pops	81%	Φ_{PT}	0.190	0.001
9) 17 populations as 8 groupings (NE; CT ; Hudson ; DE/CB ; CF/WB ; S-C/LM; E-S-O; Alt)	Among groupings	15%	Φ_{RT}	0.145	0.001
	Among pops within groupings	3%	Φ_{PR}	0.034	0.001
	Within pops	83%	Φ_{PT}	0.175	0.001
10) 17 populations as 9 groupings (#9 with SJ as a grouping)	Among groupings	15%	Φ_{RT}	0.152	0.001
	Among pops within groupings	2%	Φ_{PR}	0.025	0.001
	Within pops	83%	Φ_{PT}	0.173	0.001
11) 16 populations as 8 groupings (#10 with SJ omitted)	Among groupings	15%	Φ_{RT}	0.148	0.001
	Among pops within groupings	2%	Φ_{PR}	0.025	0.001
	Within pops	83%	Φ_{PT}	0.169	0.001

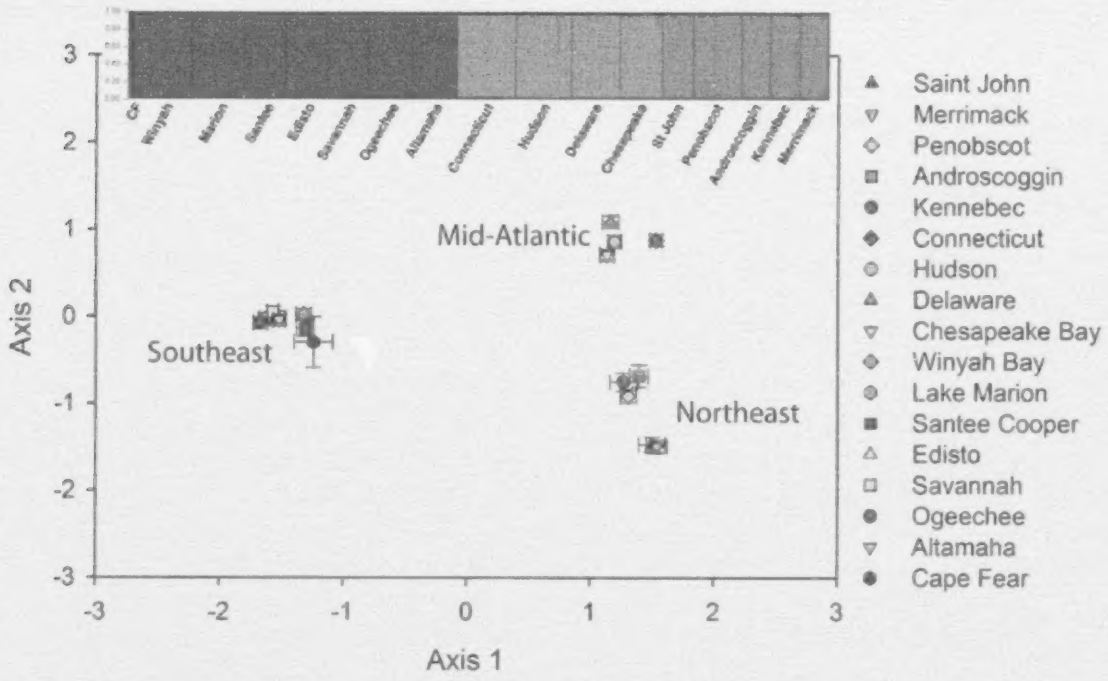

Figure 3. Principal coordinate (scatter plot) and STRUCTURE analyses (bar plot at top) of 561 Shortnose Sturgeon from 17 locations, surveyed at 11 polysomic microsatellite DNA loci. For the STRUCTURE plot, each individual is represented by a single vertical bar, broken into k colored segments, the length of which is proportional to the membership fraction in each of the k clusters. Black lines partition the river samples. (reproduced from King et al. 2014).

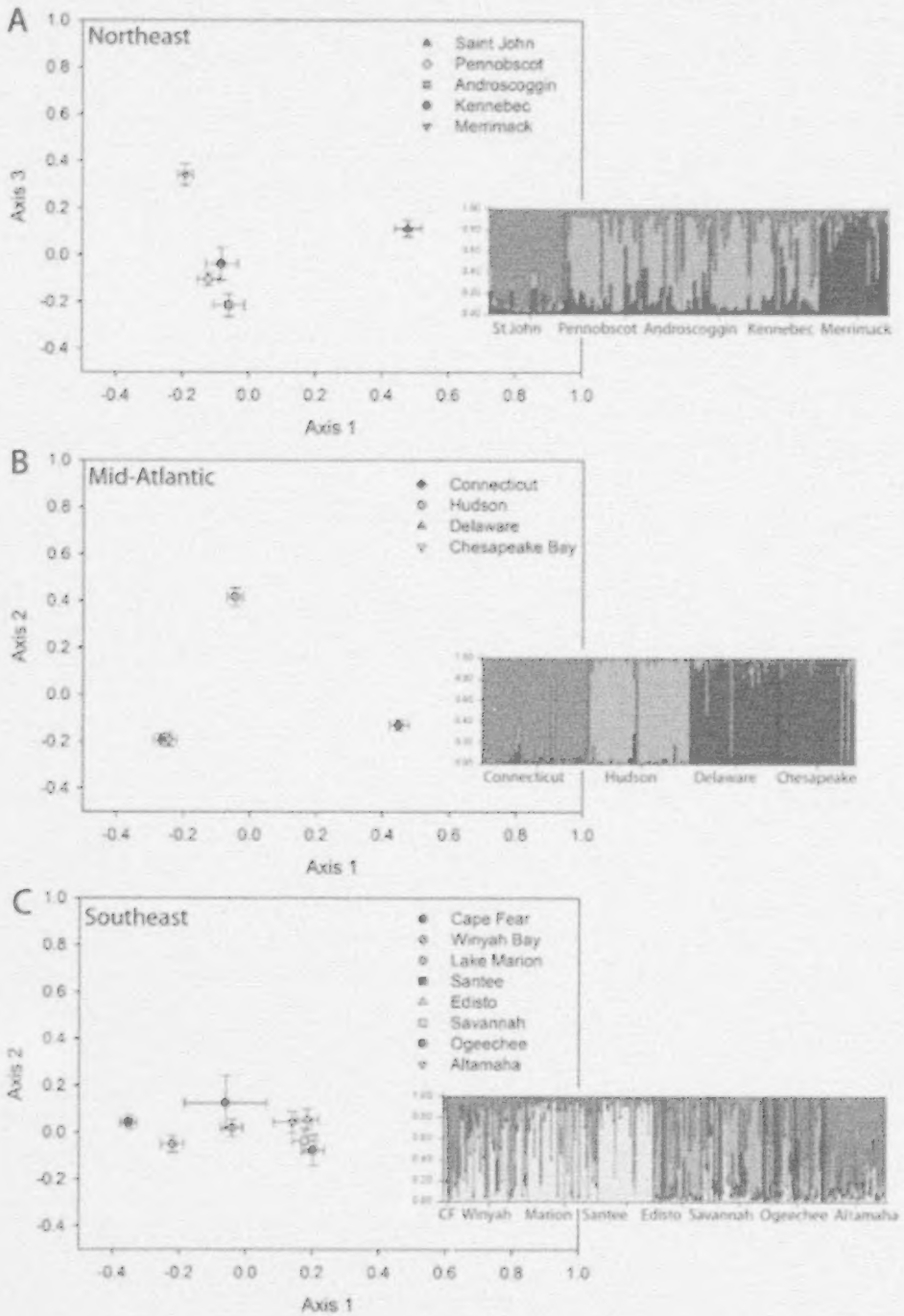

Figure 4. Sequential principal coordinates (scatter plots) and STRUCTURE (bar plots) analyses of 561 Shortnose Sturgeon surveyed at 11 polysomic microsatellite DNA loci. For the STRUCTURE histograms, each individual is represented by a single vertical bar, broken into k colored segments, the length of which is proportional to the membership fraction in each of the k clusters. Black lines partition the river samples. A) Northeast region collections; B) Mid-Atlantic region collections; C) Southeast region collections. (reproduced from King et al. 2014).

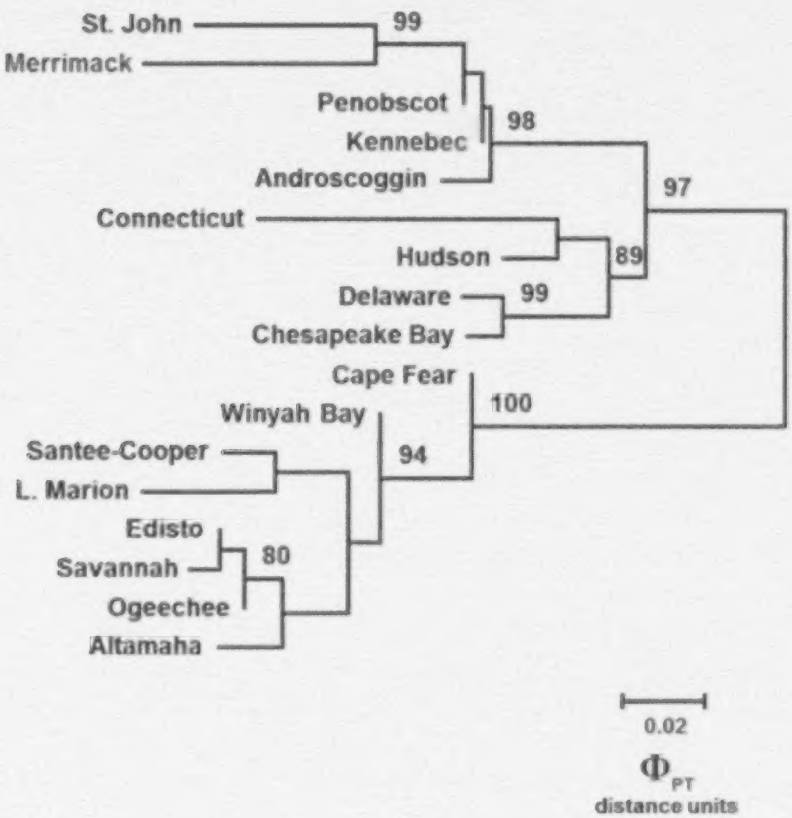

Figure 5. Dendrogram based on Φ_{PT} distance matrix for 17 collections of Shortnose Sturgeon surveyed at 11 polysomic microsatellite DNA loci and calculated using the Neighbor-Joining method. (reproduced from King et al. 2014).

Comparison of Results from mtDNA and Microsatellites

Both King et al. (2014) and SSSRT (2010) compared the results of the mtDNA and microsatellite studies (SSSRT 2010 used a then unpublished version of the King et al. microsatellite analyses). Overall, there was a very high level of concordance in the results of the analyses conducted on the two types of genetic markers. King et al. (2014) found that the nuclear Φ_{PT} and the mitochondrial Φ_{ST} were strongly correlated ($r = 0.84$, $P < 0.0001$). Further, parallel PCoAs conducted by King et al. (2014) on the two data sets showed qualitatively similar patterns of population clustering, in which the three major population groups, Southeast, Mid-Atlantic and Northeast were clearly evident (Figure 6). However, notwithstanding the overall similarity of the two PCoAs, there were notable differences. Two such differences were the greater relative separation of the Southeast population group from the other two population groups in the PCoA based on nuclear markers, and the greater relative dispersion of populations (average distances among populations) in the mtDNA-based analysis. Such differences are attributable to a variety of factors that distinguish the mtDNA and microsatellite data, such as different inheritance mechanisms (maternal vs. biparental), different numbers of independent genetic markers (1 vs. 11), and likely levels of homoplasy (low vs. high). On the other hand, a notable similarity between the two PCoAs was the relatively large separation between the SJR and other populations in the Northeast group.

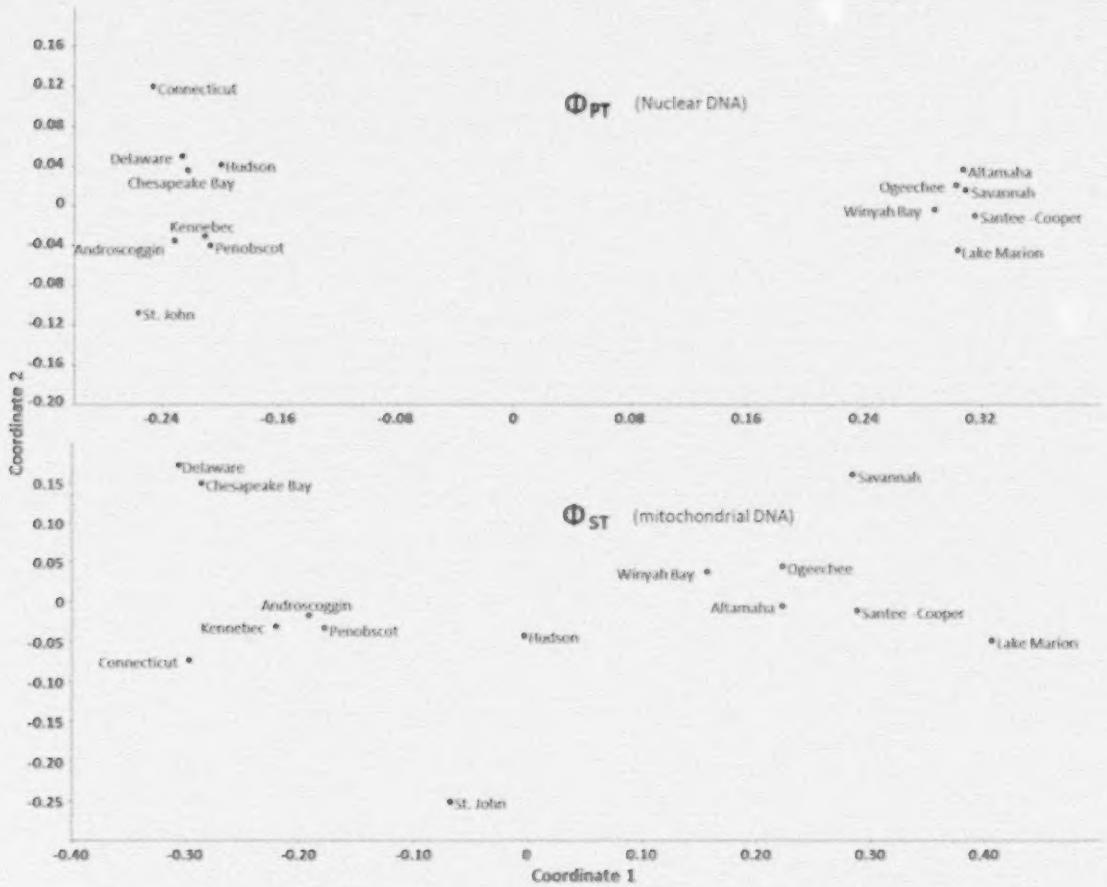

Figure 6. Results of independent multidimensional scaling analyses of pair-wise a) Φ_{PT} (nuclear DNA microsatellites) and b) Φ_{ST} (mitochondrial DNA; Wirgin et al. 2010) matrices for 14 Acipenser brevirostrum collections that are in common between the two studies. (reproduced from King et al. 2014).

Conclusions: Evidence Bearing on *Discreteness* and *Significance*

As noted above, the approach used in both the US and Canada to determine which units of intra-specific biological diversity merit recognition for potential legal protection is to apply a two-part test that first assesses *discreteness*, then *significance*. *Discreteness* is commonly assessed using neutral genetic markers: if populations show statistically significant differences in the frequency of neutral genetic markers, then they are *discrete*. Both the mtDNA and microsatellite data provide strong evidence of *discreteness* for the SJR Shortnose Sturgeon population, since both mtDNA haplotype and nuclear microsatellite genotype frequencies differ significantly between the SJR and the nearest populations to the south in Maine.

Significance is often a more challenging criterion to ascertain, and this is true for Shortnose Sturgeon. Three possible types of evidence that could support significance that involve ecological or distributional issues (see Table 1) are outside the scope of this report. The fourth type of evidence relies on genetic data. Here, the Canadian guidelines are more explicit, and, therefore, potentially more restrictive, than the US guideline. The US guideline requires only that the DPS "differs markedly from other populations"; whereas, the Canadian instructions refer to "deep intraspecific phylogenetic divergence" and "fixed differences" and further state that

"quantitative (frequency) differences for rapidly-evolving markers, such as microsatellites, generally would not be sufficient...". On the other hand, COSEWIC deliberately uses the term 'guidelines' to allow some flexibility in the interpretation of data. Moreover, recent COSEWIC practice in recognizing DUs, particularly in marine and anadromous fishes, has demonstrated such flexibility (e.g., COSEWIC 2010a,b). Another line of evidence that could support significance, although not explicitly referred to in the COSEWIC guidelines, would be genetic data that provide evidence of local adaptations. Such data are currently rare or non-existent for most species, but are likely to rapidly become widely available with ongoing advances in DNA sequencing technologies.

The Final Recovery Plan for the Shortnose Sturgeon recognized 19 DPSs, one of which was the SJR (NMFS 1998); however, this report preceded publication of any relevant genetic data. Since then, three published reports have presented opinions on the number of Shortnose Sturgeon distinct population segments that are supported by the genetic data. The SSRT report (SSSRT 2010) weighed both the mtDNA and microsatellite data and concluded that the relatively deep divisions among the three major population groups supported by both the mtDNA and nuclear DNA data "likely delineate evolutionarily significant differentiation and adaptive potential for this species". Further, the authors decided that the "narrow zones of genetic discontinuity" seen in the mid-Atlantic region (for example in the second round Structure analyses) were also likely to be of biological significance for the species. Therefore, they concluded that there are five Shortnose Sturgeon regional population clusters in the US. These comprise "U.S. (Gulf of Maine, Connecticut/Housatonic rivers, Hudson River, Delaware River/Chesapeake Bay, and Southeast) *plus the Saint John population cluster in New Brunswick, Canada*" (italics added). The phrasing is slightly ambiguous, but it implies that the SJR would have been recognized as a 'regional population cluster' if it were in the U.S.

Wirgin et al. (2010) concluded from a consideration of their mtDNA data that "there are no less than nine genetically discrete Shortnose Sturgeon populations at this time". In reaching this conclusion, they lumped together the Penobscot and Kennebec (including the Androscoggin tributary), as well as the Delaware and Chesapeake, and Cooper and Santee, Ogeechee and Savannah, but split all other rivers. In this interpretation, the SJR was regarded as a genetically discrete population.

Finally, King et al. (2014) also evaluated both the mtDNA and microsatellite data sets and decided that the number of "demographically discrete and evolutionarily significant lineages" was most likely seven, based on their evaluation of both their nuclear microsatellite and the Wirgin et al. (2010) mtDNA data. These seven groups consisted of the following combinations: (i) Southeast (all rivers south of Chesapeake Bay); (ii) Delaware-Chesapeake; (iii) Hudson; (iv) Connecticut; v) Merrimack; (vi) Androscoggin, Kennebec, Penobscot; and (vii) Saint John River.

Thus, three sets of authors, some overlapping between reports (T. King and M. Kieffer are authors on both the SSSRT (2010) and King et al. (2014) publications), reached somewhat different conclusions regarding the number of 'genetically discrete' or 'evolutionarily significant' Shortnose Sturgeon populations, but all acknowledge the genetic distinctiveness of the SJR Shortnose Sturgeon population.

Because there is no firm biological or quantitative definition of what constitutes a DPS, there will inevitably be scope for contention regarding the DPS status of the SJR. Nonetheless, on the basis of the results outlined above, a strong argument can be made that the SJR Shortnose Sturgeon population merits recognition as a DPS. Likewise, and particularly considering recent precedents set by COSEWIC (2010a,b), the SJR population would likely be considered a DU on the basis of COSEWIC guidelines and recent practice.

Population Status

Shortnose Sturgeon are found on the east coast of North America from New Brunswick, Canada, to Florida, US. The only known Shortnose Sturgeon population in Canada is found in the Saint John River, in southwest New Brunswick (Figure 1). The first published record of Shortnose Sturgeon in the Saint John River was in 1957 (Leim and Day 1959). Prior to this, they were likely not distinguished from Atlantic Sturgeon, which support a commercial fishery. There is no information on the status of SJR Shortnose Sturgeon prior to construction of Mactaquac Dam, which was built in the mid-1960s about 15 km upstream of Fredericton and was opened in 1968. There has been only a single comprehensive census of the Shortnose Sturgeon population in the Saint John River below the Mactaquac Dam, and it was conducted in the 1970s (Dadswell 1979).

Shortnose Sturgeon was evaluated as Special Concern by COSEWIC in 1980, and it was re-assessed and evaluated as Special Concern in 2005. According to COSEWIC, Shortnose Sturgeon met the criterion for Threatened based on criterion D2 (i.e., Canadian population with a very restricted index of area of occupancy or number of locations, based on presence in only one river) but were designated as Special Concern because there were considered to be no immediate threats to the population. SJR Shortnose Sturgeon are to be reassessed by COSEWIC in April 2015. Threats to SJR Shortnose Sturgeon are not known to have increased since the COSEWIC (2005) assessment.

Abundance and Distribution of SJR Shortnose Sturgeon

Dadswell 1979

A comprehensive mark-recapture study of SJR Shortnose Sturgeon was conducted between June 1973 and June 1977 by Dadswell (1979). Shortnose Sturgeon were captured primarily with gill nets (though some were captured incidentally in salmon trap nets). Sampling was weekly, except from July 1976 to June 1977. From 1973-1976, Carlin tags were applied externally to healthy specimens (adults and juveniles). A gill net survey of the summer and winter distribution pattern in the estuary was conducted in 1973 and early 1974. Placement of gill nets within each embayment was by random-grid plan. During July and August 1975, sets were made over the entire estuary according to a random-grid plan. During the last week of June 1976 and of June 1977, intensive recapture efforts were made in Grand Bay, a location where Shortnose Sturgeon were concentrated at the time. Marking of unmarked fish during the final two efforts was by caudal fin clipping. In each winter of the study period, gill nets were fished under the ice in Grand Lake, Washademoak Lake, Belleisle Bay, Kennebecasis Bay, and Long Reach. Small numbers of Shortnose Sturgeon that were captured in salmon trap nets at Westfield between 1973 and 1976 were also examined and marked. In total, 4,178 sturgeon were captured: 2,453 of these were tagged and released, 330 of these were recaptured, and 1,153 were released untagged.

Average adult population estimates ranged from 6,200 to 18,500, rising from lower values during the early part of the study period to higher values (18,500±4,600, 17,700±5,900 and 17,700±6,200) during the last three recapture cycles. Initial estimates were considered to be low because repeated sampling in the same locations during 1974 resulted in higher recapture rates and depressed the population estimates. The last three estimates, based on more widespread and randomized sampling, were considered likely to be closer to the true adult population level at the time. In subsequent reports, Dadswell (1984) describes the adult Shortnose Sturgeon population as 18,000±30%, with a total Canadian population of 100,000 through extrapolation of the mortality relationship.

This study identified overwintering locations within the large, deep lakes of the lower Saint John River drainage and deep saline portions of the river and lower estuary (i.e., Grand Lake, Washamadoak Lake, Bellisle Bay, Kennebecasis Bay, and Long Reach), as well as in Bay of Fundy waters adjacent to the Saint John River. It also described the seasonal movements of Shortnose Sturgeon, including upriver migrations of mature Sturgeon in spring. Spawning was inferred in the region of the river between the Mactaquac Dam and Fredericton (Dadswell 1984). Over-wintering locations are also described in Dadswell et al. (1984).

Litvak (1998-2004)

Mark-recapture studies were conducted on fish provided by participants in recreational angling derbies directed on sturgeon that occurred during October in the Kennebecasis River from 1998 to 2004. Average annual abundance was estimated as 2,068 fish (801-11,277) using a Jolly-Seber open population estimate technique (COSEWIC 2005). High interannual variability in census population size has been attributed to variable immigration and emigration from other tributaries of the Saint John River. The data are considered to reflect a persisting population (given the long-term annual catches in the sturgeon derby), but it is not considered sufficiently precise to infer change in overall abundance with time(reference).

Li et al. 2007

The objectives of this study were to locate a Shortnose Sturgeon overwintering site, discover how Shortnose Sturgeon use their habitat in winter, and estimate their population size.

In this study, three Shortnose Sturgeon caught in the Kennebecasis River (September 2004) were surgically implanted with acoustic transmitters (VEMCO) were and tracked to their overwintering location at the confluence of Kennebecasis River and the Hammond River (Figure 7) using a directional hydrophone (Sep-Oct 2004). The overwintering habitat use of sturgeon in this area was then investigated from January to March 2005 (when on-ice conditions were safe) using an underwater video camera system and habitat modeling approach. Holes (187 of them) were drilled in ice, and a video camera was dropped through the holes. Counts of Shortnose Sturgeon at each hole were recorded, and habitat type was noted.

A total of 234 Shortnose Sturgeon were counted. They appeared to use the deepest part of the sandy substrate and did not appear to move much during the sampling period. The total abundance estimate from this study was 4,836±69 fish using the ordinary kriging method to interpolate sturgeon density at unsampled sites. This estimate was thought to represent an unknown portion of the total population, because it represents only one of the several known historical overwintering locations (see Dadswell 1979, 1984).

Interestingly, the Kennebecasis overwintering site discovered by Li et al. (2007) had not been identified by Dadswell (1979, 1984). Fish carrying acoustic transmitters (including these three and others not discussed in the paper) did not travel to the sites identified by Dadswell (1979, 1984). Nor was the Murphy Cove site identified by Dadswell verified via video when surveyed during 2007. However, given the potential small size of overwintering sites, thus the difficulty in detecting them, this does not necessarily mean that sites in these areas are not currently used by Shortnose Sturgeon for overwintering.

Usvyatsov et al. 2012

Following on work by Li et al. (2007), the densities and overall abundances of overwintering Shortnose Sturgeon were estimated using underwater video at the Kennebecasis River over-wintering site in 2009 and 2011. The objectives of this study were to characterize the year-to-year variability in Shortnose Sturgeon numbers aggregating at the site, the accuracy and precision of measurements taken using the underwater camera–laser system, and the length distribution of overwintering Shortnose Sturgeon. Sampling was conducted from Feb-March

2009 (8 days) and Feb 2011 (5 days) at approximately the same location as was sampled by Li et al. (2007).

In total, 362 and 222 overwintering Shortnose Sturgeon were counted at 186 and 144 holes (0.02 km^2 area) during 2009 and 2011, respectively. The majority of Shortnose Sturgeon were observed on sandy substrate, similar to Li et al. (2007). Estimates of the fork lengths of 83 Shortnose Sturgeon sampled using the camera-laser system ranged from 54 -119 cm (76.6±13.7 cm), which suggests fish in the range of 11-57 years (adults). This was compared to the fork lengths of 44 Shortnose Sturgeon captured in the October 2008 Kennebecasis River sturgeon derby (79.8±10.3 cm), which were estimated to be between 14 and 33 years.

Three different models of spatial fish density estimated a total of 3,852–5,222 adult Shortnose Sturgeon in 2009 and 2011. For 2009 results, exponential, spherical, and Gaussian models gave estimates of 4,083.3±50.5, 4,769.2±48.7, and 4,450.3±48.1 Shortnose Sturgeon, respectively. For 2011 results, exponential, spherical, and Gaussian models gave estimates of 3,852.1±92.9, 5,163.8±90.8, and 5,222.4±90.3, respectively. No juveniles were observed at this site, which indicates they were overwintering elsewhere.

Like the Li et al. (2007) study, the intent of this study was primarily to identify this as important freshwater habitat for Shortnose Sturgeon. The intent was not to establish a population estimate. Attempts were made by Usvyatsov (Litvak, pers. comm.) to find overwintering Shortnose Sturgeon at South Bay (RKM12), Washademoak Lake (RKM65), and Gagetown (RKM72) using ultrasonically tagged fish during the winter of 2011 and by dropping cameras into these locations. Shortnose Sturgeon were tracked to an area in the SJR near Gagetown, 5 km upstream of a site identified by Dadswell (1979), and they were observed aggregating there in low densities (Litvak, pers. comm.).

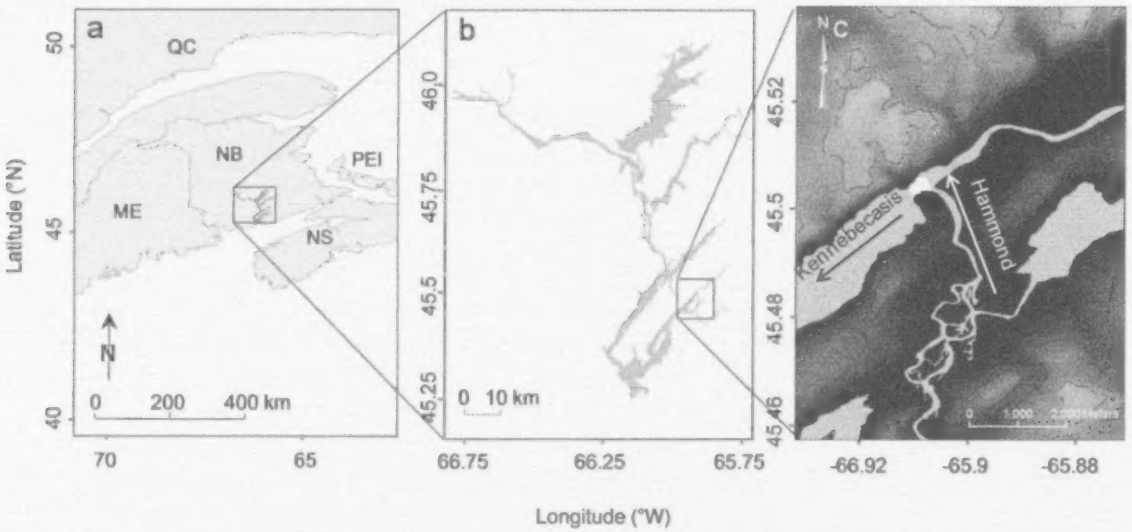

Figure 7. (a) The Atlantic coast, showing the Maritime Provinces of Canada (NB = New Brunswick; NS = Nova Scotia; PEI = Prince Edward Island), as well as Quebec (QC) and Maine (ME); (b) the lower Saint John River and the Kennebecasis River (rectangle indicates the sampling site); and (c) the Kennebecasis–Hammond River confluence, where overwintering Shortnose Sturgeon were sampled (arrows = direction of river flow; white polygon = sampling site). (reproduced from Usvyatsov et al. 2012)

Size Distribution and Growth Rates of SJR Shortnose Sturgeon

Trends in size (age) distribution and growth rates can be used to inform population status, with changes (e.g., truncation of older ages) potentially being related to changes in the environment or to human impact (e.g., removals).

Dadswell et al. (1984) provided a size distribution plot for Shortnose Sturgeon caught in gillnets in the Saint John River during 1974 and 1975 (Figure 8). He also provided a corresponding age distribution plot (Figure 9). These plots indicate that the broad size range sampled reflected a broad age spectrum, with age classes up to at least 40 years well represented.

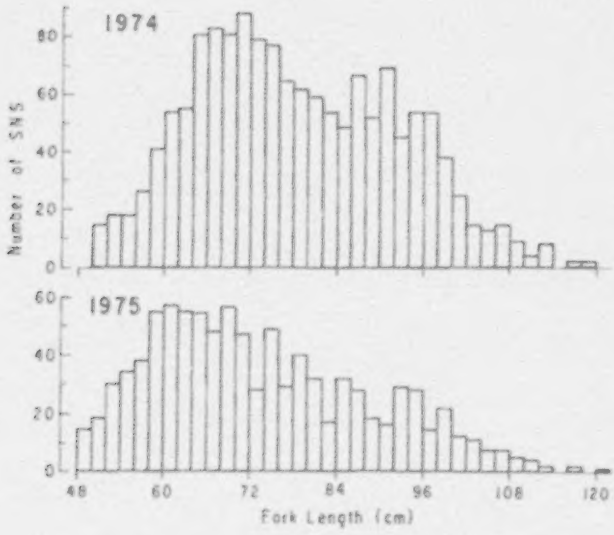

Figure 8. Fork length size composition gill net catches of Shortnose Sturgeon caught in the Saint John River in 1974 and 1975 (1973 also provided in the report but not shown here) (Dadswell et al. 1984).

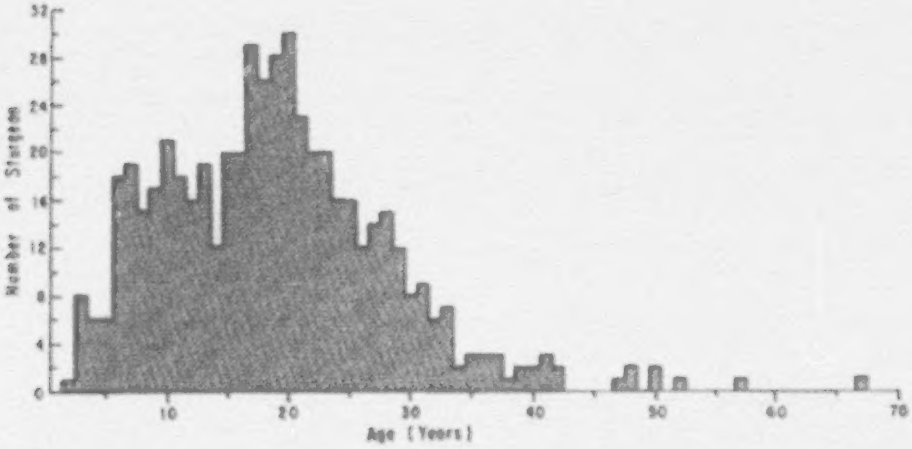

Figure 9. Age composition of Shortnose Sturgeon sampled from the Saint John River. Predominance of fish around age 20 is an artifact of gill net selectivity for that size of sturgeon. Fewer Shortnose Sturgeon of younger age reflects small amount of effort with nets selective for that size and the differential distribution of juveniles and adults (Dadswell et al. 1984).

Litvak (COSEWIC 2005) provided a size distribution plot for Shortnose Sturgeon caught in gillnets in the Saint John River during 1998-2002 (Figure 10). This shows a similar broad size distribution to the plot from the 1970s. While there is no corresponding age distribution plot, it is assumed that this broad size spectrum also reflects a broad distribution of ages.

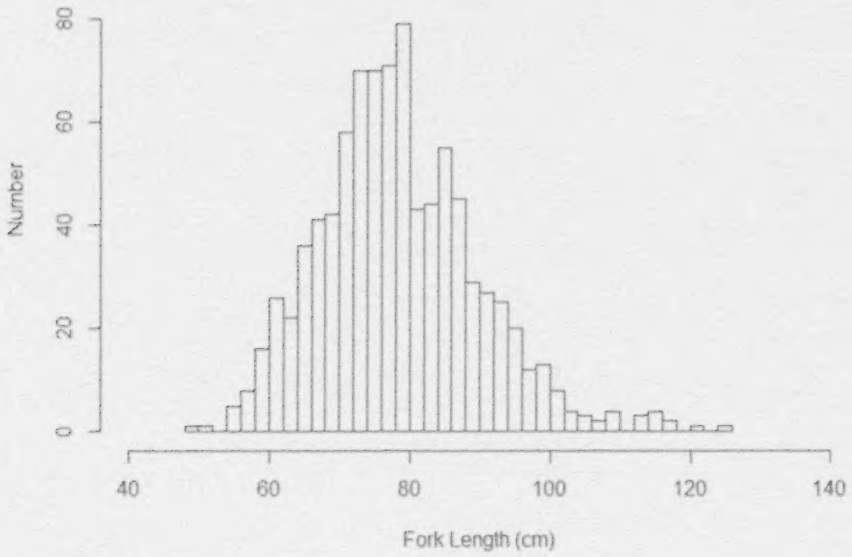

Figure 10. Fork length frequency distribution of Shortnose Sturgeon caught in the Saint John River using a 12.5 cm stretch gill net (1998-2002). The current size limit for retention in the recreational fishery is any fish that is at least 120 cm fork length.

Dadswell (1979) estimated growth rates from 106 tagged recaptured fish between 1973 and 1978. During this period, Shortnose Sturgeon were found to gain, on average, 490 grams per year (1.32 g/day). Shortnose Sturgeon in the Saint John River (1998-2002) had an average annual mean weight gain of 540 g (gaining 1.48 g per day $\pm$1.2 SE) or a specific growth rate (following Ricker 1975) of 0.017% $\pm$ 0.067 per day (COSEWIC 2005).

Minimum Viable Population Size and Carrying Capacity

In 2014, Stokesbury et al. (2014) reviewed the potential use of the Species Ability to Forestall Extinction (SAFE) index to evaluate the status of Shortnose Sturgeon on the east coast of North America (the main focus of the paper was on Atlantic Sturgeon).

In this paper, the Shortnose Sturgeon adult population estimate for the east coast of North America was 96,775. Most of this population estimate was from the Hudson River (Bain et al. (2007) provided an estimate of about 57,000 adults based on data collected from 1994-1997), followed by the Saint John River (using the 1979 estimate of 18,000 adults, which was assumed to be at carrying capacity).

The minimum viable population threshold for Shortnose Sturgeon on the east coast of North America was assumed to be 5,000 individuals based on Traill et al. (2010). This value was based on a literature review of a variety of vertebrate (mammal) species, few of which were fish.

The SAFE Index (Clements et al. 2011) was calculated as:

$$SAFEindex = \log_{10} N - \log_{10} MVP_t$$

where N was equal to the species total population estimate throughout its range (all populations combined) and MVP_t was the assumed minimum viable population threshold. This gave an estimated SAFE Index for Shortnose Sturgeon of 1.29, which was considered to be above the expected value for a threatened species (0 or lower given an MVP of 5,000 adults).

In this report, the carrying capacity of the Saint John River was also estimated. The estuarine surface area of the Saint John River below the Mactaquac Dam was estimated to be 9,180 hectares (from head of tide to approximately 25 psu), which, assuming that the 1979 population estimate of 18,000 adults was at carrying capacity (an assumption that has not been tested), gives a river-specific carrying capacity of 0.51 adults per hectare. This estimate for the Saint John River was similar to the estimate for the Delaware River (0.64) but less than half of that for the Hudson River (1.29).

Summary

A recent review by Stokesbury et al. (2014) suggests that a minimum viable population size of 5,000 adults is appropriate for Shortnose Sturgeon on the east coast of North America, given their life-history characteristics and assumed low mortality (natural and fisheries-related); however, this is based on comparisons with primarily non-fish species.

A study conducted in the 1970s estimated a population size of 18,000±30% adult Shortnose Sturgeon in the Saint John River, with an estimated total population size of about 100,000 individuals with a broad size and age distribution. At this time, overwintering of Shortnose Sturgeon was reported in 5 freshwater lakes and estuaries, two locations in the lower estuary of the Saint John River (Long Reach, and just before the Reversing Falls), and in Bay of Fundy waters adjacent to the Saint John. This was considered to be a large population of Shortnose Sturgeon (the largest known at the time).

There have been no comprehensive surveys of the SJR Shortnose Sturgeon population since the 1970s, and there has been no attempt to assess the presence of Shortnose Sturgeon in the area of the Saint John River lying above Mactaquac Dam in the years since the dam became operational in 1968. Studies of a single overwintering location (the confluence between Kennebecasis and Hammond rivers; not reported as an overwintering location in Dadswell 1979), have produced estimates in the range of 4,836±69 (2005), and 3,852–5,222 (2009, 2011) adults. While these partial estimates are informative, they are not readily comparable to the historic estimates because of differences in the location, methodology, and timing of the surveys, which makes tracking of population changes over time difficult. However, these studies do indicate inter-annually persistent use of the Kennebecasis area for overwintering by a large number (in the range of 3,852–5,222) of Shortnose Sturgeon. Limited attempts to find Shortnose Sturgeon at the other overwintering locations reported in Dadswell (1979) have not been successful except for confirmation of a low density aggregation at Gagetown. However, given the potential small size of overwintering sites, thus the difficulty in detecting them, this does not necessarily mean that sites in these areas are not currently used by Shortnose Sturgeon for overwintering.

Based on sampling from 1998-2002, the size distribution of Shortnose Sturgeon remains similar to that in the 1970s and the average growth rates of SJR Shortnose Sturgeon are similar or have increased.

In summary, while information on the current population size and distribution of Shortnose Sturgeon in the SJR has not been updated, thus the trend in population size and distribution since 1979 is not known, there is no indication that the general status of the SJR Shortnose Sturgeon has changed since the 1970s. Shortnose Sturgeon were assessed as Special Concern by COSEWIC (2005) due to their distribution being limited to a single river in Canada and the lack of immediate threats to the population.

Conclusions

As there is no firm biological or quantitative definition of what constitutes a distinct population segment, there will inevitably be scope for contention regarding the distinct population segment status of SJR Shortnose Sturgeon. Nonetheless, on the basis of the results outlined above, a strong argument can be made that the SJR Shortnose Sturgeon population merits recognition as a distinct population segment.

A study conducted in the 1970s estimated a population size of 18,000±30% adult Shortnose Sturgeon in the Saint John River. Subsequent studies at a single overwintering location have produced estimates in the range of 4,836±69 (2005), and 3,852–5,222 (2009, 2011) adults. While these partial estimates are informative, they are not readily comparable to the historic estimates because of differences in the location, methodology, and timing of the surveys. However, these studies do indicate persistent use of the Kennebecasis area for overwintering by a large number (in the range of 3,852–5,222) of adult Shortnose Sturgeon. In addition, size distribution and growth rates of Shortnose Sturgeon sampled in the Saint John River from 1998-2002 appear to be similar to those reported in 1979. Thus, while information on the current population size and distribution of Shortnose Sturgeon in the SJR has not been updated, thus the trend in population size and distribution since 1979 is not known, there is no indication that the general status of the SJR Shortnose Sturgeon has changed since the 1970s. Shortnose Sturgeon were assessed as Special Concern by COSEWIC (2005) due to their distribution being limited to a single river in Canada but acknowledging that there were no immediate threats to the population.

Research Recommendations

An updated population census for Shortnose Sturgeon in the Saint John River would provide information on the trend in population abundance and distribution since the 1970s. This could potentially be achieved through additional efforts to locate and characterize the overwintering sites for Shortnose Sturgeon in the Saint John River; for example, by ultrasonically tracking of Shortnose Sturgeon throughout the Saint John River system in late fall to locate winter aggregation sites and then surveying these sites in winter. Should Shortnose Sturgeon not be detected above Mactaquac Dam, the amount of habitat loss consecutive to the establishment of the dam could also be estimated.

Collection and analysis of new Shortnose Sturgeon genetic material could be used to calculate effective population size (the basis of genetic diversity in the population). Effective population size, or more specifically the ratio of population abundance to effective population size, could be important for determining conservation status. However, it should be noted that the genetic analysis of Shortnose Sturgeon is complicated by the fact that this species is hexaploid (i.e., contains six homologous sets of chromosomes) and long-lived. Comparison of fish from different generations will be challenging.

A directed search for Shortnose Sturgeon above Mactaquac Dam could help to more properly define the range, status, and use of the river by this species, and gain a better understanding of the potential impacts of the presence and operating regime of the dam on sturgeon population dynamics.

Additional research recommendations related to Shortnose Sturgeon will be included in the Canadian Shortnose Sturgeon Management Plan.

Contributors

Paul Bentzen	Dalhousie University
Tana Worcester	DFO Science (Maritimes)
Rod Bradford	DFO Science (Maritimes)
Simon Nadeau	DFO Science (NHQ)
Ljubica Vuckovic	DFO International Fisheries (NHQ)
Kim Robichaud-Leblanc	DFO Species at Risk Management
Greg Stevens	DFO Resource Management
Jeff Cline	DFO Resource Management
Marc Johnston	New Brunswick, DAAF
Mary Sabine	New Brunswick, DNR
Kathryn Collet	New Brunswick, DNR
Steve Gordon	New Brunswick, DNR
Jessica Pruden	NOAA
Matthew Litvak	Mount Allison University
John Waldman	Queen's College (US)
Mike Dadswell	Acadia University

Approved by

Alain Vezina
Regional Director of Science, DFO Maritimes Region
Dartmouth, Nova Scotia
Ph. 902-426-3490
Date: August 26, 2014

Sources of Information

Bain, M.B., N. Haley, D.L. Peterson, K.K. Arend, K.E. Mills, and P.J. Sullivan. 2007. Recovery of an US endangered fish. PLos, 2:e168 doi: 101371/journal.pone.0000168 2007.

COSEWIC. 2005. COSEWIC assessment and update status report on the shortnose sturgeon *Acipenser brevirostrum* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vi + 27 pp.

COSEWIC. 2010a. COSEWIC assessment and status report on the Atlantic Cod Gadus morhua in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xiii + 105 pp.

COSEWIC. 2010b. COSEWIC assessment and status report on the Atlantic Salmon Salmo salar (Nunavik population, Labrador population, Northeast Newfoundland population, South Newfoundland population, Southwest Newfoundland population, Northwest Newfoundland population, Quebec Eastern North Shore population, Quebec Western North Shore population, Anticosti Island population, Inner St. Lawrence population, Lake Ontario population, Gaspé-Southern Gulf of St. Lawrence population, Eastern Cape Breton population, Nova Scotia Southern Upland population, Inner Bay of Fundy population, Outer Bay of Fundy population) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xvii + 136 pp.

Clements, G.R., C.J.A. Bradshaw, B.W. Brooks, and W.F. Laurance. 2011. The SAFE index: Using a threshold population target to measure relative species threat. Fron. Ecol. Environ. 9:521-525.

Dadswell, M.J. 1979. Biology and population characteristics of the shortnose sturgeon, 1359 *Acipenser brevirostrum*, LeSueur 1818 (Osteichthyes: Acipenseridae), in the 1360 Saint John River Estuary, New Brunswick, Canada. Can. J. Zoology 57: 2186-2210.

Dadswell, M.J. 1984. Status of the shortnose sturgeon, *Acipenser brevirostrum*, in Canada. Canadian Field-Naturalist 98:75-79.

Dadswell, M.J., B.D. Taubert, T.S. Squiers, D. Marchette and J. Buckley. 1984. Synopsis of biological data on shortnose sturgeon (*Acipenser brevirostrum*, LeSueur 1818). NOAA Tech. Rept. NMFS 14. 45 p.

Fay, J.J. and M. Nammack. 1996. Fish and Wildlife Service's Division of Endangered Species. USA Federal Register, Department of the Interior, Fish and Wildlife Service, National Marine Fisheries Service, National Oceanic and Atmospheric Association, Commerce. Vol. 61, No. 26.

Grunwald, C., J. Stabile, J.R. Waldman, and I.I. Wirgin. 2002. Population genetics of shortnose sturgeon *Acipenser brevirostrum* based on mitochondrial DNA control region sequences. Molecular Ecology 11:1885–1898. doi:10.1046/j.1365-294X.2002.01575.x

King, T.L., A.P. Henderson, B.E. Kynard, M.C. Kieffer, and D.L. Peterson. 2013. A nuclear DNA perspective on delineating fundamental units of management and evolutionary (sic) significant lineages in the endangered shortnose sturgeon. Final Report to the National Capital Region. U.S. National Park Service and Eastern Region. USGS. 67pp.

King, T.L., A.P. Henderson, B.E. Kynard, M.C. Kieffer, D.L. Peterson, A.W. Aunins, and B.L. Brown. 2014. A nuclear DNA perspective on delineating evolutionarily significant lineages in polyploids: The case of the endangered Shortnose Sturgeon (*Acipenser brevirostrum*). PLoS ONE 9(8): e102784. doi:10.1371/journal.pone.0102784 2014

Li, X.H., M.K. Litvak, and J.E. Hughes Clarke. 2007. Overwintering habitat use of shortnose sturgeon (Acipenser brevirostrum): defining critical habitat using a novel underwater video survey and modeling approach. Can. J. Fish. Aquatic Sci. 64(9): 1248-1257.

Leim, A.H., and L.R. Day. 1959. Records of uncommon and unusual fishes from eastern Canadian waters, 1950-1958. J. Fish. Res. Board Can. 16:503-514.

[NMFS] National Marine Fisheries Service. 1998. Recovery Plan for the Shortnose Sturgeon (*Acipenser brevirostrum*). Prepared by the Shortnose Sturgeon Recovery Team for the National Marine Fisheries Service, Silver Spring, Maryland. 104 pages.

Rodzen, J.A., and B. May. 2002. Inheritance of microsatellite loci in the white sturgeon (*Acipenser transmontanus*). Genome 45:1064-1076.

Stokesbury, K.D.E., M.J.W. Stokesbury, M.T. Balazik, and M.J. Dadswell. 2014. Use of the SAFE Index to Evaluate the Status of a Summer Aggregation of Atlantic Sturgeon in Minas Basin, Canada, and the Implication of the Index for the USA Endangered Species Designation of Atlantic and Shortnose Sturgeons. Reviews in Fisheries, Science and Aquaculture 22(3):193-206.

[SSSRT] Shortnose sturgeon status review team. 2010. A biological assessment of shortnose sturgeon (*Acipenser brevirostrum*). Report to National Fisheries Service, Northeast Regional Office. November 1, 2010. 417 pp.

Traill, L.W., B W. Brook, R.R. Frankham, and C.J.A. Bradshaw. 2010. Pragmatic population viability targets in a rapidly changing world. Biol. Conserv. 143: 28–34.

[USFWS and NMFS] US Fish and Wildlife Service and National Marine Fisheries Service. 1996. Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. Federal Register 61: 4722.

Usvyatsov, S., J. Watmough, and M.K. Litvak 2012. Age and population size estimates of overwintering shortnose sturgeon in the Saint John River, New Brunswick, Canada. Trans. Amer. Fish. Soc. 141(4):1126-1136.

Waldman, J.R., C. Grunwald, J. Stabile, and I. Wirgin. 2002. Impacts of life history and biogeography on genetic stock structure in Atlantic Sturgeon, *Acipenser oxyrinchus oxyrinchus*, Gulf sturgeon Conserv Genet A. oxyrinchus desotoi, and shortnose sturgeon, A. brevirostrum. J Appl Ichthyology 18:509–518. doi:10.1046/j.1439-0426.2002.00361.x

Wirgin, I., C. Grunwald, J. Stabile, and J.R. Waldman. 2010. Delineation of discrete populations segments of shortnose sturgeon *Acipenser brevirostrum*, based on mitochondrial DNA control region sequence analysis. Conserv Genet. 11:689-708.

Wirgin, I., C. Grunwald, E. Carlson, J. Stabile, D.L. Peterson, and J.R. Waldman. 2005. Range-wide population structure of shortnose sturgeon *Acipenser brevirostrum* based on sequence analysis of the mitochondrial DNA control region. Estuaries 28:406-421. doi:10.1007/BF02693923.

This Report is Available from the:

Center for Science Advice (CSA)
Maritimes Region
Fisheries and Oceans Canada
PO Box 1006, 1 Challenger Drive
Dartmouth, Nova Scotia
Canada B2Y 4A2

Telephone: 902-426-7070
Fax: 902-426-5435
E-Mail: XMARMRAP@dfo-mpo.gc.ca
Internet address: www.dfo-mpo.gc.ca/csas-sccs/

ISSN 1919-3769
© Her Majesty the Queen in Right of Canada, 2014

Correct Citation for this Publication:

DFO. 2014. Distinctiveness and Status of the Saint John River Population of Shortnose Sturgeon (*Acipenser brevirostrum*). DFO Can. Sci. Advis. Sec. Sci. Resp. 2014/043.

Aussi disponible en français :

MPO. 2014. Distinction et situation de la population d'esturgeons à museau court (Acipenser brevirostrum) de la rivière Saint-Jean. Secr. can. de consult. sci. du MPO. Rép. des Sci. 2014/043.